Effects of three low temperature plasmas on early events of Arabidopsis thaliana germination

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The effects of 3 specific cold plasma setups have been analysed on early steps of germination of Arabidopsis thaliana seeds. We used two atmospheric pressure plasma jets using He and Ar carrier gas. We also used afterglow low pressure discharge involving N_2 with and without small O_2 concentration. Interesting efficiency attributed more particularly to reactive oxygen species has been shown on early germination in the case of plasma treated seeds during several minutes (15 min at atmospheric pressure and 20 min at low pressure).

1. Introduction:

In the field of low temperature plasmas applied to science of life, there are many research works in plasma medicine since about a decade (see e.g. [1] and references cited therein). However, there are only few investigations devoted to their use in plant biology on more particularly plasma-induced seed germination and growth. The reader can find for instance one of the first studies on plasma-induced germination and sprout growth for certain grain crops seeds [2] using a low pressure (0.1 or 0.2 Torr) DC and pulsed glow air discharges The reader can also find a recent work [3] on plasma-induced growth of tomato seeds and their bacteria resistance to avoid tomato wilt after the plant growth using low pressure (about 1 Torr) He RF discharge. More generally, several kinds of seeds (maize, oat, wheat, radish, tomato, bean, lentil, safflower, honey clover and soy, etc.) were already studied in the literature using different plasma setups (RF discharges, microwave discharges, gliding discharges, surface discharges, etc.) at low or atmospheric pressures for various gas compositions (Air, Ar, He, O_2, etc.). Even though interesting improvements of germination and plant growth were already reported, it is clear that a better understanding of the plasma species responsible of such effects and the associated mechanisms require much more works.

The present work is a first step to better understand the effect of the plasma species on the early events of germination of Arabidopsis thaliana seeds that were chosen because their genome is completely sequenced thus allowing us future investigations on the plasma-induced mechanisms. Three low temperature plasma setups were used in order to have a variety of generated plasma species:

-a dielectric Barrier Discharge (DBD) generating a pulsed plasma jet using He carrier gas at atmospheric pressure [4]

-a double DBD generating a pulsed plasma jet using Ar carrier gas at atmospheric pressure [5]

-a microwave afterglow at low pressure (a few Torr) using N_2 and O_2 mixtures [6].

Next sections are first devoted to a short description of the plasma setups and the experimental protocol used for seeds preparation, exposure to plasma and germination conditions. This is followed by some results and discussions on plasma-induced germination of seeds.

2. Materials and methods:

2.1 Low temperature plasma setups

The 3 plasma setups are already described in details elsewhere [4,5,6] and are summarized hereafter.

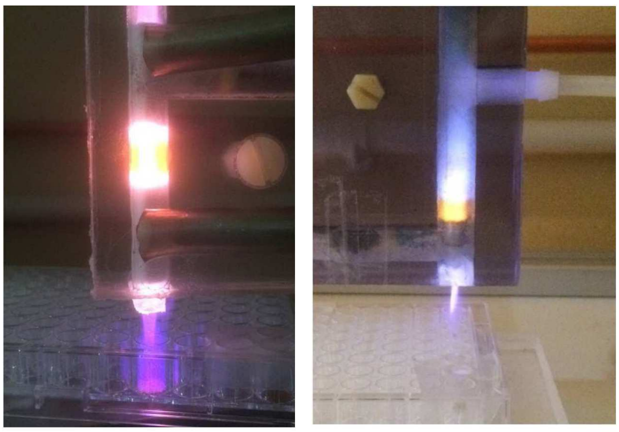

Figure 1: 5 mg of seeds inside a well of ELISA plate exposed to low temperature plasma jets at atmospheric pressure powered by pulsed voltage.
Left side: DBD configuration with He carrier
Right side: Double DBD using Ar carrier

The two setups at atmospheric pressure both generate low temperature plasma jet using as carrier gas either helium or argon at fixed gas flow (3slpm) crossing a small diameter quartz tube. The specific electrode configuration and quartz tube size for each setup are already described [4,5] with the used high voltage power source (about 9 kV, 9.69 kHz and 1µs pulse duration). These two plasma jets are displayed in figure 1 while table 1 gives some plasma characteristics.

Carrier gas	Tjet °C	Ne cm^{-3}	Te eV	[O] ppm	NOγ or OH(A-X) emission	Ions	Metastable	Ecr kV/cm
He	< 30	$\approx 10^{13}$	≈1.7	< 50	yes	He^+, He_2^+ N_2^+	$He(2^1S)$, $He(2^3S)$, $N_2(A^3\Sigma_u^+)$ $O_2(^1\Delta g)$	4.9
Ar	< 30	$\approx 10^{15}$	≈4.7	50	yes	Ar^+, Ar_2^+ N_2^+	$Ar(^4S)$ $N_2(A^3\Sigma_u^+)$ $O_2(^1\Delta g)$	12.2

Table 1: Characteristics of the plasma jet when arriving at the level of seeds inside the well (Tjet= Plasma jet temperature, Te= electron temperature, Ecr= critical field of gas rare diluted in air for 15% of dilution)

Table 1 displays an overview on the variety of species involved at the level of the seeds in the well bottom (about 3 cm from the tube outlet) where gas rare dilution in ambient air reaches about 15%. Noting that electron density, electron energy, critical electric field and more generally ionization degree are higher in the case of Ar plasma jet due to the specific configuration of the anodic high voltage electrode which is a thin wire along the quartz tube axis. Noting also the presence of metastable and ion species in the plasma that impacts the seeds during their treatments. Furthermore, the long lived excited species of more particularly He which behaves as an energy tank, can directly or indirectly favour, between two high voltage pulses (where there is no energetic electrons), the production of reactive oxygen species (ROS) as for instance singlet oxygen states able to efficiently react with seeds.

The third plasma is a microwave flowing afterglow operating at reduced pressure (5 Torr) and room temperature, already characterized and known for its bactericidal properties (Figure 1) [6,7]. Seeds were exposed to three different gas mixtures (pure N_2, $N_2/5\%O_2$ and $N_2/8\%O_2$) at constant flow rate (1 slpm) and constant injected microwave power (100 W). According to previous works [6,7], these mixtures were chosen because of their antibacterial efficiency and their ability to contain in their late afterglow region respectively a high N-atom concentration, UV radiation (due to the NOβ emission at 320 nm, consecutive to the N + O three-body recombination) and a high O-atom concentration (Table 2). Obviously in the late afterglow, there are neither charged particles nor electromagnetic field.

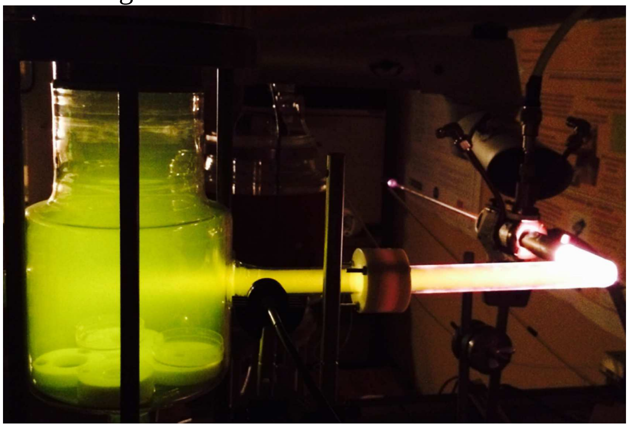

Figure 2: Picture of the post-discharge set-up showing the microwave (surfatron) discharge (right side) and the post-discharge reactor used for seed treatments (left side)

	N (cm^{-3})	O (cm^{-3})	I_{320nm} (a.u.)
Pure N_2	$1.1\ 10^{15}$	$4.0\ 10^{13}$	0
$N_2/5\%O_2$	$1.0\ 10^{14}$	$2.5\ 10^{15}$	126
$N_2/8\%O_2$	$< 10^{13}$	$4.7\ 10^{15}$	0

Table 2: N and O atoms concentrations (measured by NO titration) and UV intensity (NOβ, 320 nm) in the late afterglow regions of the three N_2/O_2 mixtures used in this study

2.2 Plant material and germination conditions

5 mg of dry seeds of *A. thaliana* (corresponding to about 200 seeds) have been used per treatment during various times (5min to 20 min). Each treatment has been duplicated. Dry seeds have been treated during various times using ELISA plate and 5-cm Petri dishes for DBD plasma jets (5 to 15 min) and low pressure afterglow (20 min) respectively.

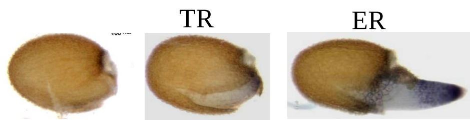

Figure 3: *A. thaliana* early steps germination following seeds imbibition. TR: testa rupture and ER: endosperm rupture (taken from [8]). NB: the seed size is about 0.5 mm length.

Seeds were sown in 5-cm Petri dishes containing half concentrated MS medium in 0.8 % agar with 1.5 % sucrose. Seeds were stratified in darkness at 4°C for 2 days and grown at 24°C in continuous light conditions. Testa rupture (TR) and endosperm rupture (ER) were quantified 24h and 30 h after transfer to light (Figure 3). Seeds treated with gas flow and sterilized seeds have been also analysed in order to detect potential effects of the gas flow without plasma (He or Ar or mixtures N_2+O_2) and to obtain a reference for the effect of the plasma respectively.

3. First results and discussion:

Germination is controlled by external factors, such as temperature, water, light and by hormone balance. Recently, reactive oxygen species (ROS) have also been shown to have a role during early steps of germination. ROS could act as messengers in addition to their role as cell wall loosening factor [8].

Ar and He plasmas largely enhanced the rate of TR and ER after 24 h and 30 h (Figure 4). The effect of the two different plasmas on both ruptures (TR and ER) is proportional to the duration of the plasma treatment.

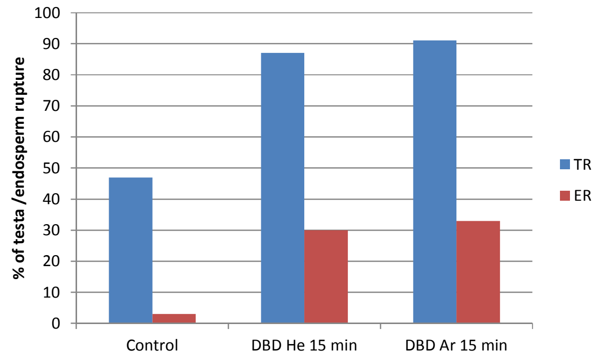

Figure 4: Effect of two different cold plasmas on *A. thaliana* seed germination rate. Germination was evaluated after 30h following treatment with DBD He and DBD argon. TR: testa rupture and ER: endosperm rupture.

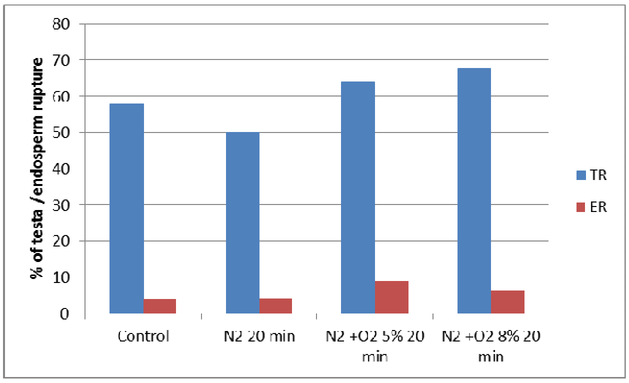

Figure 5: Effect of low pressure cold plasmas on *A. thaliana* seed germination rate. Germination was evaluated after 30h following treatment with pure N_2 afterglow and N_2 with two O_2 concentrations (5 and 8%).

Low pressure plasma with N_2 has no effect on TR and ER independently of the duration of the treatment. In contrast, afterglow plasma using mixtures of N_2 with 5% or 8% O_2 enhance the TR and ER ruptures after 24 h and 30 h. These low pressure experiments performed without and with O_2 concentrations is a clear confirmation of the role of ROS during the early germination steps.

Future analyses are necessary, more particularly in the case of atmospheric plasma jets where there are numerous active species, to determine which ROS are efficient and where and if plasma treatments affect long term plant growth such as hypocotyl and root growth.

4. Conclusion

The two atmospheric pressure plasma jets have shown quasi-similar efficiency on seed germination even if they do not involve the same active plasma species. Indeed, Ar plasma jet have higher ionization degree, atomic oxygen density, electron density, electron energy and electric field while He plasma jet requires lower ignition field and involves more long lived excited species synonymous of more reactivity between two high voltage pulse.

The reduced pressure microwave post discharge has shown the most interesting results in the case of the $N_2/8\%O_2$ mixture containing the higher concentration of atomic oxygen while pure N_2 has no effect on germination. This means that oxygen is certainly one of the species that contribute to improve of the germination.

Next step is to confirm the plasma-induced germination effects and to extend the study towards the post-germination stage of Arabidopsis thaliana seeds by analyzing the plasma effects during the plant growth.

5. References

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